

The present invention also provides an improved method of controlling plant pathogens comprising the application of an anti-pathogenic agent to plants, characterised in that plants to which the said agent is applied, are plants according to the present invention.

The gene sequences of the present invention may be synthesised *ab initio*, using the sequence data in the sequence listing provided herewith, or isolated from a library using the standard techniques known within the art. The sequences depicted in the sequence listing or parts thereof may also be used to create oligonucleotide probes for the purposes of isolating from the library those polynucleotides which are capable of producing the desired proteins. To assist the isolation of these polynucleotides we have deposited with the National Collection of Industrial & Marine Bacteria, St. Machar Drive, Aberdeen, UK, a cDNA library of the banana peel ripening related genes. The library was deposited on 9th July 1996 and has the NCIMB Accession Number 40814.

Thus, this invention is based on the identification of genes which encode proteins involved in banana ripening-related processes, specifically within banana pulp. DNA sequences according to the sequence listing or those sequences obtainable from the deposited library, may be used in the process of modifying the plant ripening characteristics of plants and/or fruit.

By virtue of this invention banana plants can be generated which, amongst other phenotypic modifications, may have one or more of the following fruit characteristics: improved

resistance to damage during harvest, packaging and transportation due to slowing of the ripening and over-ripening processes; longer shelf life and better storage characteristics due to reduced activity of degradative pathways (e.g. cell wall hydrolysis); improved processing characteristics due to changed activity of proteins/enzymes contributing to factors such as: viscosity, solids, pH, elasticity; improved flavour and aroma at the point of sale due to modification of the sugar/acid balance and other flavour and aroma components responsible for characteristics of the ripe fruit; modified colour due to changes in activity of enzymes involved in the pathways of pigment biosynthesis (e.g. lycopene, β -carotene, chalcones and anthocyanins); increased resistance to post-harvest pathogens such as fungi.

The activity of the ripening-related proteins may be either increased or reduced depending on the characteristics desired for the modified plant part (fruit, leaf, flower, etc). The levels of protein may be increased: for example, by incorporation of additional genes. The additional

genes may be designed to give either the same or different spatial and temporal patterns of expression in the fruit. "Antisense" or "partial sense" or other techniques may be used to reduce the expression of ripening-related protein.

The activity of each ripening-related protein or enzyme may be modified either individually or in combination with modification of the activity of one or more other ripening-related proteins/enzymes. In addition, the activities of the ripening-related proteins/enzymes may be modified in combination with modification of the activity of other enzymes involved in fruit ripening or related processes.

DNA constructs according to the invention for gene silencing, may comprise a base sequence at least 10 bases (preferably at least 35 bases) in length for transcription into RNA. There is no theoretical upper limit to the base sequence, it may be as long as the relevant mRNA produced by the cell but for convenience it will generally be found suitable to use sequences between 100 and 1000 bases in length. When using genomic DNA as the source of a base sequence for transcription it is possible to use either intron or exon regions or a combination of both.

As a source of the DNA base sequence for transcription, a suitable cDNA or genomic DNA or synthetic polynucleotide may be used. The isolation of suitable ripening-related sequences is described above; it is convenient to use DNA sequences derived from the ripening-related clones deposited at NCIMB in Aberdeen. Sequences coding for the whole, or substantially the whole, of the appropriate ripening-related protein may thus be obtained. Suitable lengths of this DNA sequence may be cut out for use by means of restriction enzymes. The preparation of such constructs is described in more detail below.

Constructs suitable for expression of the appropriate ripening-related sequence in banana cells, may be produced using a cDNA sequence selected from the deposited library having the NCIMB Accession Number 40814 or the gene sequence as found in the chromosome of the banana plant. Recombinant DNA constructs may be made using standard techniques. In this specification modulation means an increase or decrease of the desired effect. More specifically "modulation of the ripening or tissue senescence process in plants of the genus *Musa*" means an increase or decrease in production of a ripening related protein resulting from the method as described above. For example, where an increased ripening related protein is desired, plants may be transformed according to the method as described

above and those plants exhibiting the desired effect may be selected from the population of transformants. Furthermore, it may be desirable to provide a plant with modulated ripening or tissue senescence characteristics by increasing the production of one protein and decreasing the production of another protein in the same plant. For example, a banana fruit, modified using the present method, and having decreased levels of the enzyme pectate lyase would be beneficial because pulp softening would require a substantially longer time when compared with a control. In addition to this and by increasing the levels of another ripening related protein such as an antifungal protein in the same fruit using the present method, would complement the extended life of the banana pulp with increased resistance to disease.

Increase in production of a ripening related protein includes inserting into plant material one or more copies of any of the polynucleotides described above, wherein the said polynucleotides must be capable of producing a protein and thereby increasing protein levels when compared with a control plant.

Decrease in production of a ripening related protein includes inserting into plant material one or more copies of any of the polynucleotides described above, wherein the said polynucleotides must be capable of producing an mRNA which is capable of interfering with endogenous mRNA to such a degree that the levels of translated protein are reduced when compared with a control plant.

Ripening process of plants means the process of maturing or developing .

Senescence means the progressive deterioration in function of cells, tissues, organs etc.. related to the period of time since that function commenced..

Control plant means a comparable plant used for the purposes of determining modulation of the ripening or tissue senescence process effect in plants. Specifically, in plant transformations the control plant is usually of the same species and variety as the material used before the transformation process and is grown in the same conditions, (usually with the transformant selection step modified in some way on the part of the control plant), as the transformed plants. More specifically the control plant may comprise an untransformed control plant or a transformed control plant providing it has not already been transformed with the same polynucleotide sequence as the plant material to be transformed.

"Plant material" includes plant cells and any other type of plant regenerable material.